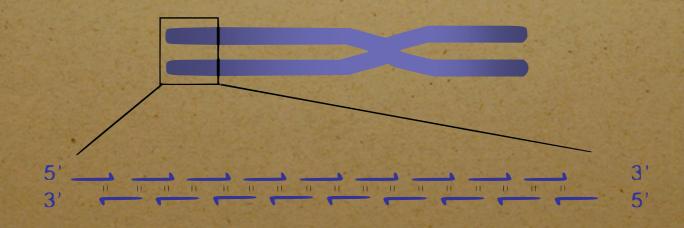
Patrick Yizhi Cai

Johns Hopkins University

Building a re-coded yeast genome powered by an army of undergraduates

Patrick Cai (蔡毅之), Ph.D.

High Throughput Biology Center Johns Hopkins University School of Medicine



Build-A-Genome

An undergrad course at Johns Hopkins

- Recoded Saccharomyces cerevisiae (SC 2.0)
 - Remove transposons
 - Cut out introns
 - Add loxPsym sites (allow genome "reshuffling" or "controllable evolution")
 - Relocate tRNA genes (hotspots for genome instability)
- End product (hopefully!): A super stable genome? A new sleek, "well-engineered" organism?

Course Setup

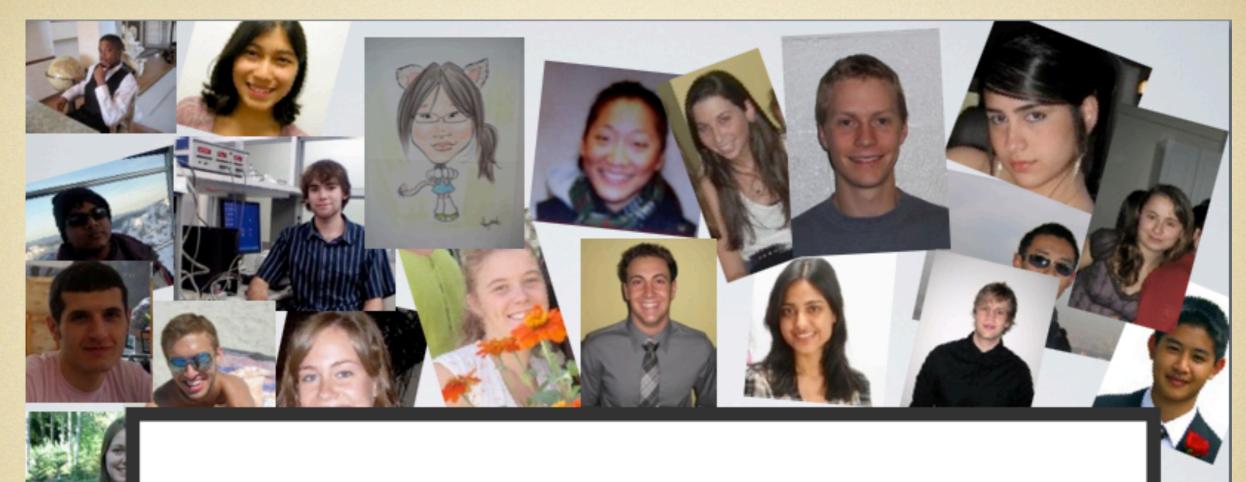
- Lectures
 - Bioinformatics
 - Genetics
 - Molecular Biology
 - Bioethics
- Bootcamp
 - Training & Milestones & Lab meetings
- Production
 - 10kb/student; Independence
- BAG Mentor

BAG Workflow

Oligo mixes Oligos distributed by instructors in 96 well plates. Students make oligo mixes for use in templateless PCR (TPCR) and finish PCR (FPCR). Templateless PCR The TPCR is performed, yielding a (TPCR) mixture of products, including the full-..... length building block from oligo mixes. Finish PCR FPCR is performed to amplify the full-(FPCR) length building block. Success of TPCR and FPCR is assessed by running small samples of FPCR on agarose gel. Cloning Building blocks determined to be of correct size are cloned into a generic **Building Blocks** cloning vector and used to transform bacteria. Colony Screening CSPCR wih vector-specific primers is PCR (CSPCR) used to determine which clones have a plasmid with insert of correct size. Sequencing Clones are sent for sequencing, and students perform sequence alignments to CCGCTTGTCCAGCGTATGC identify "perfect" clones.

Build A Genome

- Low cost undergraduate labor (i.e., FREE)
- Dinosaur equipments from Ebay (\$300 for a PCR Machine)
- Great educational value
- Open source software development
- We make available all clones, and semisynthetic yeast strains, as well as the eventual synthetic yeast strain to all who request them



Over 50 B-A-G Grads!

